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Seroprevalence of Hepatitis B and C among HIV Patients attending Wuse District Hospital Abuja, Nigeria

Obum-Nnadi C. N[1], Oyeka, C. A[2], Ezenwa C. M[3], Dennis Amaechi[4,*]

[1]Department of Microbiology, Faculty of Natural and Applied Sciences, Veritas University, Abuja, Nigeria.
[2]Department of Applied Microbiology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.
[3]Department of Microbiology, Faculty of Biological sciences, Imo State University, Owerri, Nigeria.
[4]Department of Biochemistry, Veritas university, Abuja -Nigeria.

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Abstract: Background: Human Immunodeficiency Virus and Hepatitis B Virus are among the clinical conditions of public health importance with high morbidity and mortality worldwide, especially in developing countries. These two infections share common route of transmission that puts HIV positive individuals at risk of co-infection with HBV. It is therefore necessary to document the seroprevalence of HBV among HIV patients in Abuja. Materials and Methods: The seroprevalence of HBV among HIV patients attending Wuse district hospital in Abuja was studied using the Rapid Test Detection (RTD) strips, Methods involving Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA) (PCR). Non-HIV volunteers in the same area served as control. DNA and RNA were extracted with zymo extraction kits, the genes purity quantified by Nanodrop 1000 and amplified by 9700 Applied Biosystem Thermocycler. The amplicons were resolved by Agarose Gel Electrophoresis. Phylogenetic analysis and sequencing were done by Inquaba South Africa and data analysed statistically with Graphpad prism version 7. A total of four hundred (400) subjects were involved in the study using Stratified Random Sampling method; two hundred (200) HIV patients from the district hospital and two hundred (200) for the Non-HIV volunteers within Abuja Municipal. Results: The prevalence of hepatitis B in Wuse district hospital using RTD was 4% as against 20% when PCR method was used. The phylogenetic analysis of HIV revealed HIV1 isolate closely related to AF069943.1 HIV-1 isolate with 2,538 bp genomic DNA obtained in 1995 from a hospitalized individual from Maiduguri, Borno state, Nigeria. The prevailing HBV genotype was HBV genotype E. Conclusion: The findings of this research confirm that HBV is a major co-morbid infection and a threat to HIV patients. The PCR is the best method of diagnosis. The government should improve in creating awareness and vaccinating the populace to reduce the prevalence of these infections.

Keywords: Seroprevalence; patients; phylogenetic analysis; genotype

* Corresponding author: amaechitoexcel@yahoo.com & n_charitez@yahoo.co.uk

1. Introduction

The Human Immunodeficiency Virus (HIV) poses a threat to public health. Menace with high morbidity and mortality globally, particularly in developing nations with deplorable healthcare systems. The World Health Organization (WHO) reports that, there are more than 33.4 million people living with HIV/AIDS, with over 95 percent of them residing in developing countries of which about 70% are in Sub-Saharan Africa (WHO, 2013). Hepatitis is a liver disease also implicated as a co-infection in people living with HIV/AIDS caused by hepatitis B and C viruses. Hepatitis is a leading cause of death in most developing countries including Nigeria – where the disease is misdiagnosed with malaria that share similar symptoms with hepatitis (Eze *et al*., 2014). Among the 3 viruses that share certain transmission route with HIV, the Hepatitis B and C virus infections are major public health problem worldwide (Cheesbrough, 2017). It was estimated that approximately 2 billion people (about 30% of the world's population) have serological evidence of past or present hepatitis B virus (HBV) infection. HBV infection can cause a wide range of clinical symptoms, including asymptomatic carrier status, acute hepatitis, fulminant hepatitis, chronic Hepatitis, cirrhosis of the liver (LC), and hepatocellular cancer (HCC) (WHO, 2017). Chronic hepatitis B (CHB) illness progression to severe liver diseases such as hepatocellular carcinoma (HCC) and cirrhosis (LC) is determined by host genetics, viral and environmental variables. (Biswas *et al.,* 2013; WHO, 2017). The transmission route for HBV infection can be through blood and blood products, body piercing, intravenous drug abuse, unsafe injections and sexual activity (Cheesbrough, 2017; Jafari*et al,* 2010). This puts HIV-positive people at risk of co-infection with hepatitis B, C, or both. Hepatitis C virus (HCV) infection is estimated to affect 2% of the global population. and we have about 170 million HCV carriers worldwide and is a leading cause of liver-related morbidity and mortality, most of them are thought to be in the developing countries (Cheesbrough, 2017). Despite the effective reduction in HIV/AIDS mortality and morbidity as a result of Highly Active Antiretroviral Therapy (HAART), chronic HBV and HCV infections continue to be the primary cause of death worldwide (Jafariet al, 2010). The prevalence of HIV co-infection with HCV or HBV is dependent on several factors including study population and associated-risk factors for HIV acquisition and this usually vary from region to region (Wondimeneh *et al.,* 2013). In HIV-infected individuals in 18 Sub-Saharan African nations, the prevalence of HBV and HCV was determined to be high, people ranged from 3.9-7.3 percent to 6.9 percent. Diwe *et al.,* (2013) reported HCV co-infection prevalence of 0.7%, HBV co-infection of 2.2% and no triple infection in their study in the South-East in Nigeria. This could be due to the low prevalence of intravenous drug abuse and needle sharing in the population when compared to developed countries where such practises are still prevalent. With prolonged survival of HIV infected patients, co infection with either HBV or HCV correlates with reduced survival rate. HBV and HCV have the potential to cause not only liver hepatotoxicity, but also failure of immunological recovery in HIV-positive patients, as demonstrated by a Tanzanian study that discovered a sluggish rate of immunologic recovery following commencement of HAART treatment and a higher risk of hepatotoxicity among HIV/HBV and HIV/HCV co-infected individuals was reported (Christian *et al.,* 2010). The endemicity of these infections in Nigeria necessitates the need to determine the seroprevalence of HBV and HCV infections among HIV patients in order to guide therapy and ensure proper prognosis of HIV/AIDS patients undergoing retroviral therapy in Abuja, Nigeria.

2. Materials and Methods

2.1. Study participants and sampling

A total of 200 study participants (15-75years) with CD4 count of 200 ± 490 were drawn from HIV patients attending Heart to Heart clinic in a local hospital in Abuja after obtaining an ethical approval. Control samples were drawn from additional 200 non-HIV infected individuals within the same age limit. A Stratified Random Sampling (SRS) method was used to determine the prevalence of HCV, HBsAg and anti-HBc levels from seropositive individuals in the HIV positive and control populations were calculated and expressed as a percentage. Descriptive statistics were generated for socio-demographic variables and other aspects of the sampled population. The prevalence of hepatitis in the population was described using a 95 percent confidence interval (CI). For each association, the odds ratio (OR) and confidence interval (CI) at 95 percent were calculated. A p-value < 0.05were calculated to be statistically significant.

2.2. Sample collection and processing for HIV 1/2

5 ml whole blood was collected aseptically from each participant, and was transferred to sterile test tube for separation into serum by centrifuging sample at 3000 rpm for 5 min (Ochie and Kolhatkar, 2008; Chessbrough, 2010). The Alere Determine HIV ½ (Alere Medical, USA) and chembio HIV 1/2 STAT-PAK (Chembio, USA) kits were respectively used to screen the blood samples for the detection of antibodies to HIV types 1 and 2 (HIV 1/2) in serum specimens of participants.

2.3. Screening for hepatitis B surface antigen (HBsAg)

One-Step Hepatitis B Surface Antigen Test Strip, HBsAg (Skytec Rapid Diagnostic Test, USA) and HCV Rapid Screen Test (Skytec Rapid Diagnostic Test, USA) were used for screening the blood samples for the presence of HBV and HCV antigens according to the manufacturer's instruction. The membrane of the test strip for detection of HBsAg is pre-coated with anti-HBsAg antibodies on the test line region of the strip. For HCV detection,

2.4. PCR assay for detection of HBV-DNA

DNA extraction was performed using Zymo extraction kit (Larry Jia, South Africa) according to manufacturer's instruction. HBsAg negative/positive samples of both HIV positive and HIV negative subjects was used for DNA extraction. To extract DNA, 400µl of genomic lysis buffer was added to 100µlof serum (4:1), mixed completely by vortexing for 6s, then left to sit at room temperature for 10 minutes. In a collecting tube, the mixture was transferred to a zymo-spin IIC column and centrifuged at 10,000g for one minute. The flow through collection tube was discarded, and the zymo-spin IIC column was transferred to a new collection tube containing 200l of DNA pre-wash buffer and centrifuged for 1 minute at 10,000g. The spin column was loaded with 500l of DNA wash buffer, centrifuged at 10,000g for one minute, and then transferred to a clean microcentrifuge tube. To elute the DNA, 50l of DNA elution buffer was added to the spin column, incubated at room temperature for five minutes, and then spun at maximum speed for thirty seconds. 1000 nanodrops (Thermo Fisher Scientific, Wilmington, DE, USA) is utilised for DNA quantification was used. PCR assay for amplification of HBV-DNA was performed in a final reaction volume of 20µl comprising 2µl template DNA, 4.2µl nuclease-free water, 10ul primers (Forward Primer: → 5^{I}-TCACCATATTCT TGG GAACAACA-3^{I} and Reverse Primer: 5^{I-}ACC ACT GAA CAA ATG GC-$3^{I\leftarrow}$), and 1X concentration of 10µl Taq pol, DNATPs, and MgCl. Gene

amplification was carried out in a 9700 Applied Biosystem Thermo cycler (Applied Biosystems, USA) with the PCR cycling profile consists of a 5-minute starting step at 94°C, 35 cycles of 94°C for 20 seconds, 60°C for 30 seconds, 72°C for 1 minute, and a 2-minute final extension at 72°C. Finally, 8 L of the PCR products were electrophoresed and photographed in a 1.5 percent agarose gel containing 0.5 mg/mL ethidium bromide. on an Ultraviolet Transilluminator (NRI Technologies, India).

2.5. Extraction of RNA from plasma

RNA extraction was performed using Zymo extraction kit according to manufacturer's instruction (Larry Jia, South Africa). RNA was extracted from HIV and HCV negative/positive samples of both HIV positive and negative subjects and then PCR was used for detection of HCV RNA and HIV RNA. Briefly, 300µl of viral RNA buffer was added to 100µl of plasma sample and mixed briefly by vortexing. In a collection tube, the samples were transported to the Zymo-spin IC Column and centrifuged.at 10000g for 2 min. The flow through was discarded. 500µl of viral wash buffer was added to the column and then centrifuged for 2 min at 10000g, and the column was carefully transferred into DNase/RNase-free tube. Later, 15ul of DNase/RNase free water was added to the column matrix and centrifuge for 30s to elute the RNA. Eluted RNA was stored at -70^{o}C for HIV/HCV detection.

2.6. Nested PCR for amplification of HIV V3 region

This was performed in two phases: in primary amplification, a portion of the extracted RNA was amplified by PCR using primers that flank the HIV sequence (F: →5^{I}-GGCATCAAACAGCTCCAGGCAAG-3^{I} and R: 5^{I}-AGCAAAGCCCTTTCTAAGCCCTGTCT-3^{I}←). PCR conditions included initial Denaturation at 95oC for five minutes, denaturation at 95oC for thirty seconds, and annealing at 55oC for thirty seconds extension at 72oC for 30 seconds, followed by a two-minute extension at 72oC. In secondary amplification, a portion of the HIV primary amplicon was amplified by PCR using primers that flank the HIV sequence (F: → 5^{I}-TCCTGGCTGTGGAAAGATACCTA-3^{I} and R: 5^{I}-GTCCCCTCGGGGCTGGGAGG-3^{I}←). The PCR conditions were as follows: 5 minutes of denaturation at 95°C, 30 seconds of denaturation at 95°C, 30 seconds of annealing at 55°C, 30 seconds of extension at 72°C, and 2 minutes of final extension at 72°C.

2.7. Amplification of HCV gene and sequencing

A portion of the extracted RNA was amplified by PCR using primers that flank the HCV sequence (F:→5^{I}-ACTGTCTTCACGCAGAAAGCGTCTAGCCAT-3^{I} and R:→5^{I}-CGAGACCTCCCGG GGCACTCGCAAGCACCC-3^{I}←). PCR components included a master mix (final volume was 20ul) supplied by Inqaba (Inqaba, South Africa). This comprised Taq polymerase, DNTPs, MgCl at 1X concentration and volume of 10ul; forward and reverse primers at a concentration of 0.2uM and 0.16ul volume respectively; template at 1ul volume and nuclease-free water at 8.68ul volume. Initial denaturation at 95 degrees Celsius for 5 minutes, denaturation at 95 degrees Celsius for 30 seconds, annealing at 55 degrees Celsius for 30 seconds, extension at 72 degrees Celsius for 30 seconds, and final extension at 72 degrees Celsius for 2 minutes were the PCR conditions.5 minutes of initial denaturation at 95oC, 30 seconds of denaturation at 95oC, 30 seconds of annealing at 55oC, 30 seconds of extension at 72oC, and 2 minutes of final extension at 72oC

constituted the PCR conditions. HBV, HCV, and HIV templates were sequenced on a 3510 ABI sequencer by Inqaba Biotechnological in South Africa using the BigDye Terminator kit.

2.8. Phylogenetic Analysis

The BLASTN tool was used to retrieve similar sequences from the National Center for Biotechnology Information (NCBI) database, and the acquired sequences were modified using the bioinformatics algorithm Trace edit. Clustal X was applied to align these sequences. MEGA 6.0's Neighbor-Joining approach was applied to deduce the evolutionary history. The bootstrap consensus tree constructed from 500 iterations was deemed to represent the evolutionary history of the taxa under investigation. Using the Jukes-Cantor method, evolutionary distances were estimated.

2.9. Data analysis

All data were statistically analyzed using Graphpad Prism version 7. The P values were calculated and the significant difference was also obtained. Two groups were compared using frequency analyses, Fisher's exact test, and Chi-square testing.

P-values less than 0.05 were deemed significant.

3. Result

Table 1 and 2 shows the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) among HIV/non-HIV patients attending 3 district hospitals in Abuja. Among the 200 HIV positive patients from each district hospital, 18(9%) and 182(91%) were recorded to be positive and negative for HBV in Wuse District Hospital respectively. Patients positive to HCV were 10(5%), while patients negative for the HCV were 190(95%). The prevalence of HBV and HCV were not significantly dependent on whether one is positive or negative with HIV.

Table 1. Prevalence of HBV among HIV patients attending Wuse District Hospitals and the Non HIV Volunteers

Districts	No of patients Examined	No Positive for HBsAg	No Negative for HBsAg
Wuse	200	18(9%)	182(91%)
Non HIV Volunteers	200	22(11%)	178(89%)
Total	400	4010%)	360(90%)

p>0.05

Table 2. Prevalence of HCV among HIV patients attending Asokoro District Hospitals and Non HIV Volunteers

Districts	No Examined	No Positive for HCV	No Negative for HCV
Wuse	200	10(5%)	190(95%)
Non HIV Volunteers	200	8(4%)	192(96%)

Total	400	18(4.5%)	382(95.5%)

p>0.05

The frequency of HBV among HIV/non-HIV patients attending the 3 district hospitals in Abuja is represented in Table 3. Hepatitis B surface antibody was recorded for just 35/200 (17.5%) in Wuse district, and 20/200 (10%) for non-HIV volunteers. On the other hand, no viral protein on the other hand was recorded for patients in Wuse hospitals while 9/200 (4.5%) of the non-HIV volunteers had the viral protein. The total percentage of the 400 subjects studied, was observed to be 52/400 (13%), 55/400 (13.75%), 9/400 (2.25%), 145/400 (36.25%), 155/400 (38.75%) for HBsAg, HBsAb, HBeAg, HbeAb and HbcAb correspondingly and the result is statistically significant at P<0.05.

Table 3. Prevalence of HBV among HIV patients attending the 3 District Hospitals

Districts	No of subjects	+ve HbsAg	+ve HBsAb	+ve HBeAg	+ve HbeAb	+ve HbcAb
Wuse	200	22(11%)	35(17.5%)	0(0%)	85(42.5%)	100(50%)
Non HIV volunteers	200	30(15%)	20(10%)	9(4.5%)	60(30%)	55(27.5%)
Total	400	52(13%)	55(13.75%)	9(2.25%)	145(36.25%)	155(38.75%)

p<0.05

HBV and HCV prevalence was also determined using PCR technique for patients who were not on Anti-Retroviral Therapy (ART) as represented in Table 4. It was observed that, 20/100 (20%) subjects were positive to HBsAg in Wuse District hospital. HCV positivity was found to be 19/100(19%) for Wuse District hospitals. It was also observed that 20/100(20%) and 8/100(8%) of the volunteers were positive for HBV and HCV correspondingly. The PCR and ELISA results were statistically significant at p<0.05.

Table 4. Prevalence of HBV and HCV among HIV patients attending the 3 District Hospitals and Non HIV Volunteers using PCR

Districts	No of subjects	+ve HBsAg	-ve HBsAg	+ve HCV	-ve HCV
Wuse	100	20(20%)	55(55%)	19(19%)	8(81%)
HIV Non Volunteers	100	20(20%)	80(80%)	8(8%)	92(92%)
Total	200	40(20%)	135(67.5%)	27(13.5%)	100(50%)

p<0.05

During the mega blast search for extremely similar sequences, the acquired sequence from the isolate yielded an exact match in the NCBI non-redundant nucleotide (nr/nt) database. The HB3 sequence was 99 percent comparable to other species' sequences. The Jukes-Cantor method was used to calculate evolutionary distances corresponded to the phylogenetic location of the isolates

within the Hepatitis viruses and revealed a closely relatedness to Hepatitis B virus strain SDAC_059 (gb: KF170780.1) than other Hepatitis viruses (Figure1).

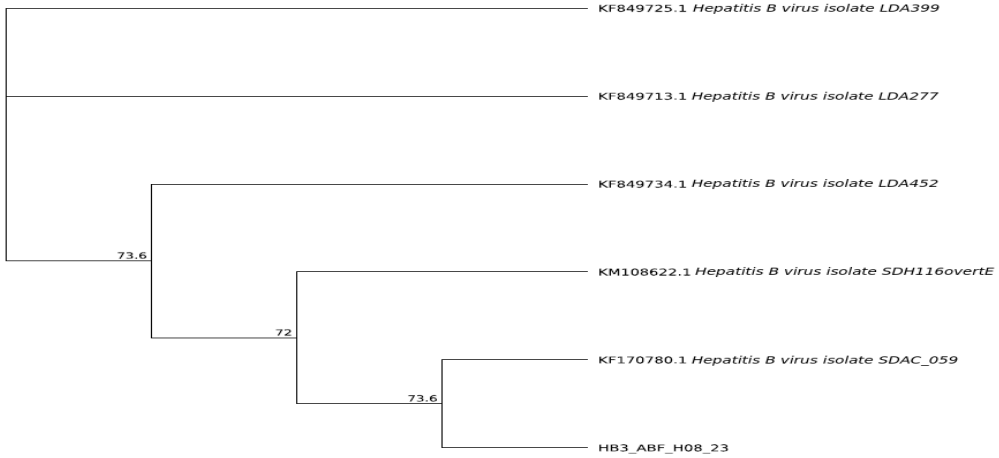

Figure 1. Phylogenetic tree showing relationship between HB3 and other Hepatitis viruses.

A total of 100 HIV samples and 100 non HIV samples positive to Hepatitis B infection, were randomly selected and were subjected to PCR-based detection for viral RNA, out of which 20(20%) samples from HIV positive patients and 20(20%) non HIV volunteers were proved as HBV positive, with a band size of approx. 600 bp in PCR, as shown in Figure 2.

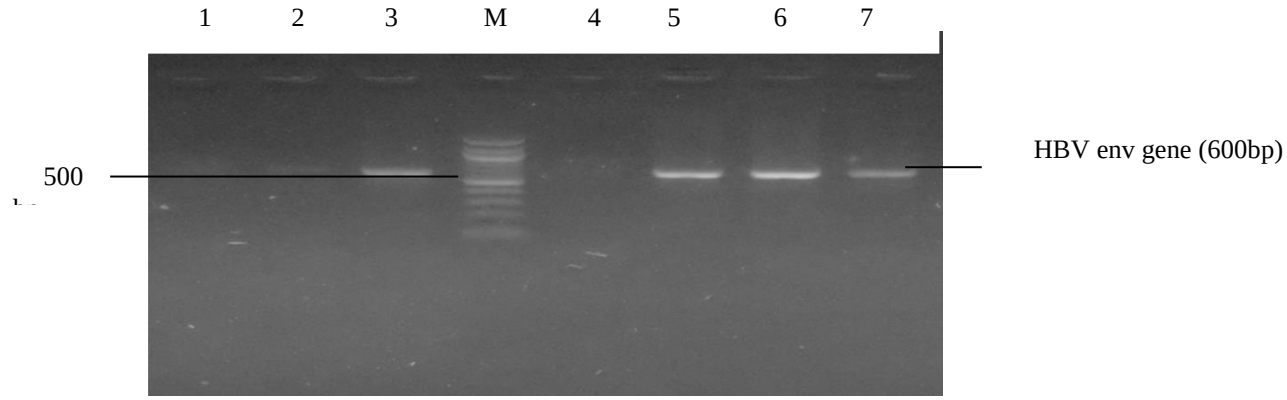

Figure 2. Plate 1. Agarose gel Electrophoresis of the HBV env gene. Lanes 3, 5, 6, 7 showing the amplified HBV env gene. Lane M represents the 100 bp ladder.

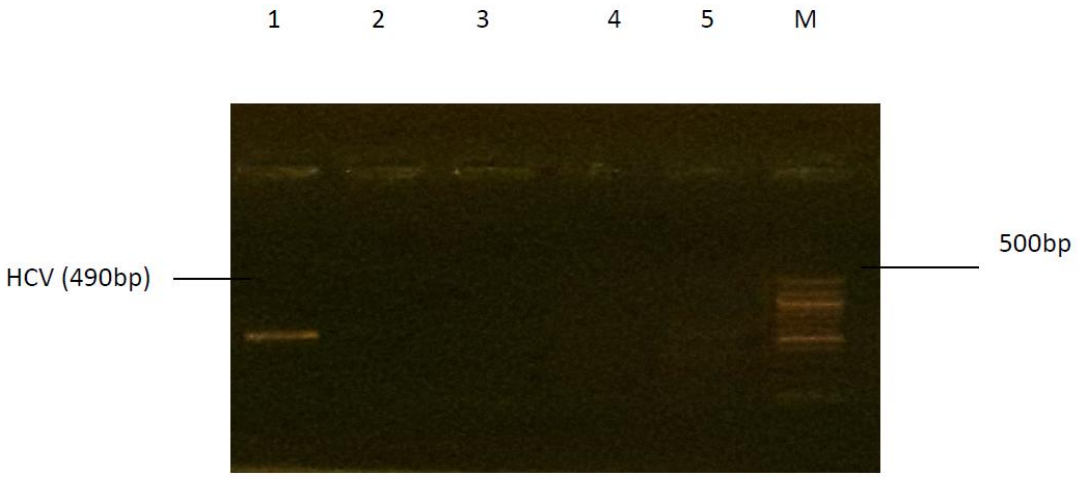

Figure 3. Agarose gel electrophoresis of amplified HCV bands; L indicates a 100bp ladder; lanes 1 and 2-5 represent HCV bands, respectively.

4. Discussion

In Nigeria, Hepatitis B, hepatitis C and HIV are common blood-borne infections that share common routes of transmission, which is unevenly distributed across different regions (Okonkwo *et al.*, 2017). A few populations based prevalence studies have been carried out to determine the prevalence of these infections and risk factors for infection with the viruses. In this study, we investigated the seroprevalence of hepatitis B and C among HIV infected individuals and various predisposing risk factors were studied. The prevalence of HBV and HCV among HIV patients attending the 3 District Hospitals surprisingly showed larger percentage of the non-HIV volunteers. This correlates with previous studies which revealed that hepatitis B infection is common than hepatitis C infection among the study group and its prevalence was considerably high even though the co-infection is at the minimal (Thompson *et al.*, 2015). A report by Atefeh *et al.*, (2015) indicated a wide range of HBV infection prevalence rate of 1.2 and 9.7% while that obtained in this research was 5% for HIV patients and 11% for non-HIV patients. The overall prevalence of HBsAg in this study population with RTD was 5%, ELISA increased to 11% and with PCR it increased to 20%. This result shows that ELISA and PCR methods gives higher prevalence and more reliable than other methods tested in this study.The prevalence rate of hepatitis B infection in Maiduguri and Ilorin was 11.6% and 5.7% (Thompson *et al.*, 2015) - which are relatively low when compared with the values obtained with PCR in this study. A study also carried out in an urban center in Nigeria showed the prevalence of hepatitis B to be 11.5%. In New York, 25% of HIV patients were co-infected with HCV and 4.4% for HBV (Diwe *et al.*, 2013), which in this case projects HCV as most prevalent infection as opposed to what was observed in this study. The prevalence of HBV and HCV are considerably high and requires that the patients be adequately attended to in order to reduce the transmission of the viral infection to other individuals and also reduce the endemicity of the infection in this part of the world. The total percentage of subjects positive to HBV and HCV across the three district hospitals both HIV positive and non-HIV positive volunteers in this present study however were 5% and 4% respectively using RTD but with PCR both the HIV infected and the non HIV infected subjects had 20% prevalence. A similar

prevalence was obtained in the work of Okonkwo *et al.,* (2017) of 8.8% prevalence for HBV, which was rated high when compared with 5.6% reported among hospital staffs and volunteers in Calabar. Based on the results obtained in this study, Nigeria can be classified as high endemic area for HBV since the prevalence is more than 8%. This agrees with the work reported earlier by other researchers that Nigeria has been classified as a hyper endemic nation for HBV with a prevalence of 12% but a relatively lower prevalence for HCV of 0.5-4% and prevalence for HIV is 3.1% (Lago *et al.,* 2014). Moderate endemicity given by WHO in 2009 for the presence of hepatitis B surface antigen (HBsAg) for HBV infection was given as 3.1%. The preventability of hepatitis B virus infection is an intriguing aspect. This can be accomplished through measures similar to those taken to prevent the transmission of HIV, such as safe sex practises, safe handling of sharps, and avoiding sharing intravenous drug paraphernalia, among others. Reflecting on the difference in prevalence rates obtained in different study on HIV/HBV/HCV around the same region and different regions of Nigeria and the world at large, the difference could be as a result of differences in sample technique, sample size, and sample population. The phylogenetic analysis of the HIV samples confirmed that the patients were all HIV patients. Genotype E is more predominant in Africa, genotype F is more common in South America and Polynesia, and genotype G is more predominant in the United States and France. Studies made by Andernach *et al.,* 2013 documented that one of the three genotypes that are predominates in Africa depending on the region includes; E, A and D. While genotype D predominates in Northern Africa, genotype A predominates in East and South Africa. Except in Cameroon, where genotype A is dominant, genotype E is widespread throughout Sub-Saharan Africa. HBV genotype E has a high prevalence and wide geographic spread throughout large parts of Africa. This is in line with findings made in this research, all the HBV isolated from the three district hospitals are of genotype E. Conclusively, this study reports a high proportion of non-HIV patients with hepatitis infection when compared to the HIV positive patients in this study. A high prevalence of hepatitis among HIV and non-HIV individuals was also recorded, and this necessitates the need for constant monitoring to evaluate control measures. The results classified Abuja as high endemic area for HBV infection in Nigeria. To offer an opportunity to identify individuals with diagnosed and undiagnosed infection, population-based screening should be conducted in the urban and rural areas of the country for HIV, HBV, HCV and other viral infection known to be endemic in the country. This will help reduce the national burden of complications resulting from these infections among HIV positive and non-HIV positive individuals alike.

References

[1] Andernach, I. E., Hunewald, O. E. and Muller C.P.(2013). Bayesan Inference of Evolution of HBV/E. *Plos One* 8(11): e81690. www.ncbi.nlm. Accessed November 29, 2013.

[2] Atefeh Y., HadisY., Sahar T., and Kiarash G. (2015). Prevalence of Hepatitis B and C among Patients Looking for Hospital Care; Five Years' Study in Mashhad, Iran. Tehran University of Medical Sciences. *Acta Medica Iranica Journals*, 2016; 54(1):54-57.

[3] Biswas A., Panigrahi R., Pal M., Chakraborty S., Bhattacharya P., Chakrabarti S., and Chakravarty R. (2013). Shift in the hepatitis B virus genotype distribution in the last decade among the HBV carriers from eastern India: possible effects on the disease status and HBV epidemiology. *Journal of Medical Virology*. 85:1340–1347.

[4] Cheesbrough M. (2010). *District Laboratory Practice in Tropical Countries Part 2*. Cambridge University Press, International Sales Department, UK. Low price. 5th edn.

[5] Christian B, Okuma J, Claudia H, Chalamilla G, Spiegelman D, and Nagu T. (2010). Prevalence of hepatitis B and C Co-infection and response to antiretroviral therapy among HIV-infected patients in an urban setting in Tanzania. California: *17th Conference on Retroviruses & Opportunistic Infections in San Francisco.*

[6] Diwe, C. K., Emmanuel C. O., Oguamanam O. E., Jerome E. A., and Nathan C. N. (2013). Sero-prevalence of hepatitis B virus and hepatitis C virus among HIV patients in a suburban University Teaching Hospital in South-East Nigeria. *Pan African Medical Journal.*16:7.

[7] Eze J. C, Ibeziako N. S, Ikefuna A. N, Nwokye I. C, Uleanya N. D, IIechukwu G. C. (2014). Prevalence and risk factors for hepatitis C and human immunodeficiency virus coinfection among children in Enugu Nigeria. *African Journal of Infectious Diseases*. 8:5–8.

[8] Jafari S, Copes R, Baharlou S, Etminan M, and Buxton J. (2010). Tattooing and the risk of transmission of hepatitis C: a systematic review and meta-analysis. *International Journal of Infectious Diseases.*14(11):928-40.

[9] Lago, B. V., Mello, F.C.,Ribas, F. S., Valente, F., Soars, C. C. Niel, C. and Gomes, S. A. (2014). Analysis of Complete Nucleotide Sequences of Angolan Hepatitis B Virus Isolates Reveals the Existence of a Separate Lineage with Genotype F. *Plos one* 9(3).

[10] Ochei, J. and kolhatker, A., (2009). Medical laboratory science. Theory and practice. Tata McGraw hill. 9th edn. Pp 185-186.

[11] Okonkwo U. C., Okpara H., Otu A., Ameh S., Ogarekpe Y., Osim H., and Inyama M. (2017). Prevalence of hepatitis B, hepatitis C and human immunodeficiency viruses, and evaluation of risk factors for transmission: Report of a population screening in Nigeria. South African Medical Journal. 107(4):346-351. DOI:10.7196/SAMJ.2017.v107i4.12198.

[12] Schmidt, (2008). Hepatitis B and C in HIV-infected patients. Journal of Hepatology. 27(1): 18-24.

[13] Thompson J. A., Tolulope A. B., Funmilayo J. A., Festus A. O., Festus O. A., Olalekan I. O. (2015). Prevalence of Hepatitis B Virus and Hepatitis C Virus Co-Infections among Ekiti People in South-Western Nigeria. International Journal of Health Sciences& Research (www.ijhsr.org). 5(3):121-126.

[14] WHO (2009). Global surveillance and control of hepatitis C. Report of a WHO consultation organized in collaboration with the Viral Hepatitis Prevention Board, Journal of Viral Hepatitis. Antwerp, Belgium. 6:35-47.

[15] WHO (2013). Global surveillance and control of hepatitis C. Report of a WHO consultation organized in collaboration with the Viral Hepatitis Prevention Board. Journal of Viral Hepatitis. Antwerp, Belgium. 6:35-47.

[16] Wondimeneh, Y., Alem, M. Asfaw, F., and Belyhun, Y. (2010). HBV and HCV seroprevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals at University of Gondar Teaching Hospital, Northwest Ethiopia. Plos One. 10:171. PMCID: PMC3670208.